et al. and further in view of **Haseltine** et al., **Kang** and **Rodman**. This rejection is respectfully traversed.

First of all, the Examiner contends that **Haseltine** et al. and **Kang** provide the complete nucleotide/amino acid sequence of the HIV-1 *tat* gene. Applicants respectfully disagree. Applicants reiterate that **Haseltine** et al. and **Kang** are not applicable as prior art because they do not teach or suggest anything about the HIV-1 *tat* gene.

Haseltine et al. disclosed HTLV-III/LAC tat_{III} gene that encoded the HTLV-III/LAV associated trans-acting factor (column 3, lines 21-23). HTLV-III/LAV is the human T cell leukemia virus III (column 1, lines 21-22). Human T cell leukemia virus III is not the same as human immunodeficiency virus type I (HIV-1) as claimed herein. These are two different viruses. The HTLV-III/LAC tat_{III} gene and the HIV-1 *tat* gene are two different genes from two different viruses. **Haseltine** et al. only taught the HTLV-III/LAC tat_{III} gene. **Haseltine** et al. did not teach or suggest the HIV-1 *tat* gene as claimed herein. Hence, **Haseltine** et al. is irrelevant as a prior art.

Kang disclosed baculovirus expression system capable of producing foreign gene proteins at high levels. **Kang** only taught the

rev (example 1), vif (example 2) and pol (example 3) proteins of HIV-1. **Kang** did not teach or suggest the HIV-1 *tat* gene. Therefore, **Kang** does not disclose anything that is relevant to the present invention.

Consequently, the relevant prior art is **Brey** et al. in view of **Georgiou** et al. and further in view of **Rodman**. **Brey** et al. disclosed attenuated strain of bacteria that express malarial antigens. **Georgiou** et al. disclosed recombinant DNAs that are suitable for the expression of heterologous antigen on the surface of an enteric microorganism. **Rodman** disclosed a natural human IgM antibody reactive against HIV-1 tat protein. Applicants submit that combining **Brey** et al., **Georgiou** et al. and **Rodman** would not lead one of ordinary skill in the art to the present invention.

The instant invention is drawn to an HIV-1 Tat-expressing attenuated bacterial host that can induce both cellular and humoral anti-HIV-1 immune responses. **Brey** et al., **Georgiou** et al. and **Rodman** did not teach or suggest an HIV-1 Tat-expressing bacteria can be used to induce anti-HIV-1 immune responses. Furthermore, neither did **Brey** et al., **Georgiou** et al. and **Rodman** teach or suggest an HIV-1 Tat-expressing attenuated bacterial host

can induce both cellular and humoral anti-HIV-1 immune responses as claimed herein.

The importance of inducing a cellular immune response for an HIV vaccine to be effective has become increasingly clear in the past few years. The CTL response exerts substantial pressure on HIV replication during both the primary and chronic stages of infections. It is postulated that if the CTL response is great enough infected cells can be killed before allowing the virus to mutate. (McMichael and Rowland-Jones 2001 (attached)) This hypothesis is supported by the consistent presence of a cellular immune response specific to HIV in highly exposed persistently seronegative (HEPS) cohorts. (Miyahira, Murata et al. 1995 (attached); Rowland-Jones, Dong et al. 1999 (attached); Kaul, Rowland-Jones et al. 2001 (attached); Rowland-Jones, Pinheiro et al. 2001 (attached); Li, Promadej et al. 2002 (attached)) Also HIV specific CTL responses have been detected in uninfected exposed health care workers and uninfected babies born to infected mothers ((Pollack, Zhan et al. 1997 (attached); Buseyne, Burgard et al. 1998(attached); Buseyne, Chaix et al. 1998 (attached); Riviere and Buseyne 1998 (attached); Wasik, Wierzbicki et al. 2000 (attached))

The Examiner contends that it would have been obvious to express the HIV-1 *tat* gene provided by **Rodman** as an Lpp-OmpA-Tat fusion protein as suggested by **Georgiou** et al. in the *S. typhimurium* expression system described by **Brey** et al. because **Brey** et al. teach that this system is useful for generating strong immune responses. However, **Brey** et al. only taught immune responses to malarial antigens. **Brey** et al. did not teach or suggest an immune response to the product of HIV-1 *tat* gene. Teaching on immune responses to malarial antigens cannot be generalized to immune responses against HIV-1 Tat protein because, *inter alia*, induction of cellular and/or humoral immune responses by a putative antigen cannot be predicated and ascertained until actual experiments are carried out in model animals. In the absence of a teaching related to inducing specific immune responses to HIV-1 Tat, the cited references do not provided one of ordinary skill in the art with the requisite expectation of successfully producing Applicants' claimed invention because induction of immune responses by HIV-1 Tat has to be determined empirically.

The Examiner also contends that the skilled artisan would have been motivated to combine the cited references to make an HIV-1 Tat-expressing bacteria because that would facilitate the

development of HIV-1 Tat-specific immunological reagents for diagnostic, immunological or biochemical assays. Applicants submit that the cited references do not teach or suggest development of HIV-1 Tat-specific immunological reagents for diagnostic, immunological or biochemical assays. Furthermore, the present invention is not related to development of HIV-1 Tat-specific immunological reagents for diagnostic, immunological or biochemical assays. The essence of the present invention is an HIV-1 Tat-expressing bacterial host that can induce both specific cellular and humoral immune responses against HIV-1. As discussed above, the cited references are deficient in teaching or suggestion related to this critical element of inducing specific anti-HIV-1 immune responses.

The Examiner further contends that "all of the components employed in the instant application have been disclosed in the cited references. Both the bacterial host and fusion protein had already been used to produce recombinant proteins. Viral transactivating proteins have been cloned, sequenced and expressed in disparate expression systems. Therefore, there was a reasonable expectation of success of sufficient motivation for combining the aforementioned references." Applicants respectfully disagree.

Initially, Applicants note that the mere fact that "all of the components employed by the applicants... were well-known in the prior art" is immaterial to a determination of obviousness under35 U.S.C. §103. It is established law that one may combine known prior art components into a novel, non-obvious composition.

Secondly, it appears that the Examiner is arguing for reasonable success in expressing viral transactivating protein as fusion protein in the disclosed bacterial host. However, the present invention is not drawn to such bacteria. The essence of the present invention is a recombinant bacterial host that can induce cellular and humoral immune responses against HIV-1. Even though it may be obvious to try to construct an HIV-Tat expressing bacterial host, it is not obvious from the combined teaching of **Brey** et al., **Georgiou** et al. and **Rodman** that such bacteria can induce both cellular and humoral anti-HIV immune responses as claimed herein. The cited references do not teach or suggest methods or reagents that can induce cellular and humoral immune responses against HIV-1. The cited references also fail to provide the requisite expectation of success because the induction of cellular and humoral immune responses against a specific antigen must be determined empirically.

See also the above discussion concerning the importance of inducing cellular immune responses.

In view of the above remark, **Brey** et al., **Georgiou** et al. and **Rodman** do not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed invention. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1, 2, and 5-11 under 35 U.S.C. §103(a) be withdrawn.

Claims 1, 2, and 5-11 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Hone** et al. in view of **Georgiou** et al. and further in view of **Haseltine** et al., **Kang** and **Rodman**. This rejection is respectfully traversed.

As discussed above, **Haseltine** et al. and **Kang** are irrelevant as prior art. Hence, the applicable prior art is **Hone** et al. in view of **Georgiou** et al. and further in view of **Rodman**.

Hone disclosed attenuated *Salmonella* vaccine vector containing expression vector encoding HIV-1 gp120 fusion protein. **Georgiou** et al. and **Rodman** have been discussed above. Applicants submit that combining **Hone** et al., **Georgiou** et al. and

Rodman would not lead one of ordinary skill in the art to the present invention.

The present invention is drawn to an HIV-1 Tat-expressing attenuated bacterial host that can induce both cellular and humoral anti-HIV-1 immune responses. **Hone** et al., **Georgiou** et al. and **Rodman** did not teach or suggest an HIV-1 Tat-expressing bacteria can be used to induce anti-HIV-1 immune responses. Neither did **Hone** et al., **Georgiou** et al. and **Rodman** teach or suggest an HIV-1 Tat-expressing attenuated bacterial host can induce both cellular and humoral anti-HIV-1 immune responses as claimed herein.

The Examiner contends that **Hone** et al. teach *Salmonella* that induce both mucosal and systemic HIV-1 gp120-specific immune responses. However, **Hone** et al. only taught antibody responses in mucosal tissue and in blood. **Hone** et al. did not teach or suggest induction of cellular immune response (e.g. T cell response) by the HIV-1 gp120 protein. Indeed, **Hone** et al. taught that the issue of inducing cellular anti-HIV-1 immune responses by *Salmonella* vaccine strain is unresolved because "presently there is no consensus on the vector configuration that optimizes the ability of *Salmonella* to induce foreign antigen-specific cytotoxic $CD8^+$ CTLs in

vivo." (page 206, left column, third paragraph). Again, note the discussion above concerning the importance of inducing cellular immune responses for development of effective HIV vaccines.

In contrast, the present application shows that an HIV-1 Tat-expressing bacterial host can induce both cellular and humoral anti-HIV-1 immune responses. Further, Applicants submit herein a Declaration that demonstrates attenuated *Salmonella* with the HIV epitope-containing plasmids can cause the induction of a cytotoxic CD8 T cell response. Applicants submit that even though various components of the present invention are disclosed in **Hone** et al., **Georgiou** et al. and **Rodman**, the cited references do not teach or suggest an HIV-1 Tat-expressing bacterial host can induce both cellular and humoral anti-HIV-1 immune responses as shown and claimed herein.

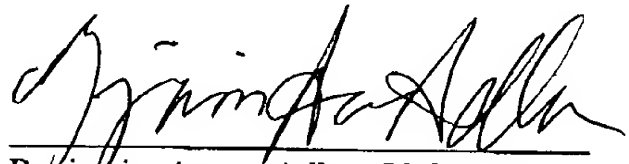
Similar to the discussion *supra* regarding **Brey** et al., **Georgiou** et al. and **Rodman**, Applicants submit that **Hone** et al., **Georgiou** et al. and **Rodman** do not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed invention in view of the lack of teaching and suggestion on possible induction of both cellular and humoral immune responses by an HIV-1 Tat-expressing

bacteria. Induction of cellular and/or humoral immune responses by a putative antigen cannot be predicated and ascertained until actual experiments are carried out in model animals. Thus, the invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1, 2, and 5-11 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Final Office Action mailed September 10, 2002. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Nov 29, 2002



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Buseyne, F., M. Burgard, et al. (1998). "Early HIV-specific cytotoxic T lymphocytes and diseas progression in children born to HIV-infected mothers." AIDS Res Hum Retroviruses **14**(16): 1435-44.

The activities of HIV-specific cytotoxic T lymphocytes (CTLs) were evaluated in 10 HIV-infected children, born to infected mothers who did not receive AZT during pregnancy. CTL activities were present as early as 4 months of age. The five children that progressed to AIDS before 1 year of age had reduced in vivo and in vitro CTL activities, when compared with children who remained AIDS free after 1 year of age. The latter children had weak in vivo activated CTL responses but strong memory CTLs. No relation was found between viral load, lymphocyte populations, and CTL responses between birth and 6 months of age. Between 7 and 12 months old, children with broader in vitro activated CTLs had higher absolute numbers of CD4+ and CD8+ T lymphocytes and lower plasma viral load. These data support a beneficial role of CTLs in pediatric HIV infection.

Buseyne, F., M. L. Chaix, et al. (1998). "Cross-clade-specific cytotoxic T lymphocytes in HIV-1-infected children." Virology **250**(2): 316-24.

We studied cytotoxic T lymphocyte (CTL) cross-reactivity between human immunodeficiency virus type 1 (HIV-1) subtypes within a group of infants infected with either HIV-1 B or non-B clade. Fifteen children were infected with a clade B virus. Nine were infected with non-B virus, including two clade A, four clade D, two clade F, and one clade G. CTL activities from in vitro activated peripheral blood mononuclear cells were tested against autologous cell line infected with recombinant vaccinia viruses encoding for Env, Gag, Pol, or Nef proteins from a clade A or B isolate. HIV-1-specific CTL elicited from infection with clade B virus could lyse targets expressing clade A proteins, and vice versa. In infants with positive CTL responses, cross- clade recognition was predominant and was detected within 88% of the Pol, 83% of the Nef, 67% of the Gag, and 55% of the Env responders. Longitudinal studies showed that CTL cross-reactivity to both B and A targets was stable for several years. Elicitation of CTL reactivities capable of elimination of virus-infected cells is an important goal for the development of an efficient AIDS vaccine. The significant cross- reactivity of CTL shown in this study supports the concept that vaccines developed using a single-clade immunogen may be applicable to induce broadly reactive T cell responses.

Kaul, R., S. L. Rowland-Jones, et al. (2001). "New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers." Immunol Lett **79**(1-2): 3-13.

A clearer understanding of HIV-1 specific immune responses in highly-exposed, persistently seronegative (HEPS) subjects is important in developing models of HIV-1 protective immunity. HIV-1 specific cytotoxic T-lymphocytes (CTL) have been described in a cohort of HEPS Kenyan sex workers, and recent work has further lucidated these responses. CTL specific for HIV-1 Env were found in the blood of over half the sex workers

meeting criteria for HIV resistance, and in some women recognized unmapped pitopes. The proportion of women with Env- specific CTL increased with the duration of uninfected HIV exposur , suggesting that these responses were acquired ov r time. CD8+ lymphocyte responses directed against predefined HIV-1 CTL epitopes from various HIV-1 genes were found in the blood and genital tract of >50% resistant sex workers, at a ten-fold lower frequency than in infected subjects. The epitope specificity of CD8+ responses differs between HEPS and HIV infected women, and in HEPS the maintenance of responses appears to be dependent on persistent HIV exposure. Several HIV-1 'resistant' sex workers have become HIV infected over the past 6 years, possibly related to waning of pre-existing HIV-specific CTL, and infection has often been associated with a switch in the epitope specificity of CD8+ responses. These findings suggest that vaccine- induced protective HIV immunity is a realistic goal, but that vaccine strategies of boosting or persistent antigen may be necessary for long- lived protection.

Li, L., N. Promadej, et al. (2002). "Crystallization and preliminary X-ray crystallographic studies of HLA- A*1101 complexed with an HIV-1 decapeptide." Acta Crystallogr D Biol Crystallogr **58**(Pt 7): 1195-7.
A major goal of vaccine research for the prevention of AIDS is to determine the immune correlates of protection against HIV-1 infection. In this context, it is of interest to understand how HLA-A*1101, a significantly more prevalent class I allele in a cohort of highly HIV-1- exposed persistently seronegative individuals, functions in relation to protective immunity to HIV-1. Towards this goal, a soluble recombinant HLA-A*1101 molecule has been expressed and used to assemble a complex with beta2-microglobulin and a Nef decapeptide. The HLA- A*1101/beta2m/Nef complex was crystallized by the hanging-drop vapor- diffusion method. The crystal formed in the monoclinic space group P2(1), with unit-cell parameters a = 77.2, b = 88.5, c = 64.8 A, beta = 90.1 degrees, and contains two molecules in the asymmetric unit. A data set to 2.2 A resolution was collected and structure determination by molecular replacement is currently in progress. Understanding the three- dimensional structure of the HLA-A*1101/beta2m/Nef complex may provide insight into the functional role of this class I allele in relation to protective immunity to HIV-1.

McMichael, A. J. and S. L. Rowland-Jones (2001). "Cellular immune responses to HIV." Nature **410**(6831): 980-7.
The cellular immune response to the human immunodeficiency virus, mediated by T lymphocytes, seems strong but fails to control the infection completely. In most virus infections, T cells either eliminate the virus or suppress it indefinitely as a harmless, persisting infection. But the human immunodeficiency virus undermines this control by infecting key immune cells, thereby impairing the response of both the infected CD4+ T cells and the uninfected CD8+ T cells. The failure of the latter to function efficiently facilitates the escape of virus from immune control and the collapse of the whole immune system.